AMENDMENTS TO THE CLAIMS

1. (withdrawn) A method for real-time detecting and quantifying a nucleic acid template in a PCR mixture comprising the steps of

a) thermally cycling the PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, the nucleic acid template, primers to amplify at least one amplicon from the nucleic acid template, and a double stranded DNA dye, wherein the amplicon has a melting temperature of T_m;

b) obtaining cycle by cycle a pre-T_m emission at a MT below the T_m and a post-T_m emission at the a MT above the T_m;

c) determining cycle by cycle an emission amount of the amplicon, which is the difference between the pre-T_m emission and the post-T_m emission.

2. (withdrawn) The method of claim 1 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

3. (withdrawn) The method of claim 2 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

4. (withdrawn) The method of claim 1 wherein the double stranded DNA dye is a primer-based double stranded DNA dye.

5. (withdrawn) The method of claims 4 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

6. (withdrawn) The method of claim 1 wherein the MT below the T_m is 0.25 ^{O}C below, 0.5 ^{O}C below, 1.0 ^{O}C below, 1.5 ^{O}C below, or 2.0 ^{O}C below the T_m.

7. (withdrawn) The method of claim 1 wherein the MT above the T_m is 0.25 ^{O}C above, 0.5 ^{O}C above, 1.0 ^{O}C above, 1.5 ^{O}C above, or 2.0 ^{O}C above the T_m.

8. (withdrawn) The method of claim 1 wherein the emission amount of the amplicon is obtained through a computer program which performs a calculation of subtracting the pre-T_m emission from the post-T_m emission or the post-T_m emission from the pre-T_m emission.

9. (original) A method for real-time detecting and quantifying a first nucleic acid template and a second nucleic acid template in a PCR mixture comprising the steps of

a) thermally cycling a PCR mixture wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first T_m and the second amplicon has a second T_m and the first T_m is less than the second T_m;

b) obtaining cycle by cycle a first emission at a first MT between an annealing/extension temperature and the first T_m and a second emission at a second MT between the first T_m and the second T_m;

c) determining cycle by cycle a first emission amount of the first amplicon which is the difference between the first emission and the second emission, and a second emission amount of the second amplicon which is the second emission.

10. (original) The method of claim 9 further comprising a step of obtaining cycle by cycle a third emission at a third MT between the second T_m and a total denaturing temperature, wherein the second emission amount is the difference between the second emission and the third emission.

11. (original) The method of claim 9 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

12. (original) The method of claim 11 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

13. (original) The method of claim 9 wherein the double stranded DNA dye is a primer-based double stranded DNA dye.

14. (original) The method of claims 13 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

15. (original) The method of claim 9 wherein the first MT is 0.25 ^{O}C below the first T_m, 0.5 ^{O}C below the first T_m, 1.0 ^{O}C below the first T_m, 1.5 ^{O}C below the first T_m, or 2.0 ^{O}C below the first T_m, and wherein the first MT is higher than the annealing temperature.

16. (original) The method of claim 9 wherein the second MT is 0.25 ^{O}C below the second T_m, 0.5 ^{O}C below the second T_m, 1.0 ^{O}C below the second T_m, 1.5 ^{O}C below the second T_m, or 2.0 ^{O}C below the second T_m, and wherein the second MT is higher than the first T_m.

17. (original) The method of claim 9 wherein the second MT is 0.25 ^{O}C above the first T_m, 0.5 ^{O}C above the first T_m, 1.0 ^{O}C above the first T_m, 1.5 ^{O}C above the first T_m, or 2.0 ^{O}C above the first T_m, and wherein the second MT is less than the second T_m.

18. (original) The method of claim 9 wherein the second MT is the first T_m + 0.25^{O}C< the second MT< the second T_m-0.25^{O}C, the first T_m + 0.5^{O}C< the second MT< the second T_m-0.5^{O}C, the first T_m + 1.0^{O}C< the second MT< the second T_m-1.0^{O}C, the first T_m + 1.5^{O}C< the second MT< the second T_m-1.5^{O}C, or the first T_m + 2.0^{O}C< the second MT< the second T_m-2.0^{O}C.

19. (original) The method of claim 10 wherein the third MT is 0.25 ^{O}C above the second T_m, 0.5 ^{O}C the second T_m, 1.0 ^{O}C above the second T_m, 1.5 ^{O}C above the second T_m, or 2.0 ^{O}C above the second T_m, and wherein the third MT is less than the total denaturing temperature.

20. (original) The method of claim 9 wherein the emission amount of the first amplicon is obtained through a computer program performing a calculation of subtracting the first emission from the second emission or subtracting the second emission from the first emission.

21. (original) A method for real-time detecting and quantifying a first nucleic acid template and a second nucleic acid template in a PCR mixture comprising the steps of:

a) thermally cycling a PCR mixture wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first T_m and the

second amplicon has a second T_m and the first T_m is less than the second T_m;

b) obtaining cycle by cycle a first pre-T_m emission at a MT below the first T_m and a first post-T_m emission at the a MT above the first T_m and a second pre-T_m emission at a MT below the second T_m and a second post-T_m emission at the a MT above the second T_m;

c) determining cycle by cycle a first emission amount of the first amplicon which is the difference between the first pre-T_m emission and the first post-T_m emission; and a second emission amount of the second amplicon which is the difference between the second pre-T_m emission and the second post-T_m emission.

22. (original) The method of claim 21 wherein the double stranded DNA dye is a double stranded DNA intercalating dye

23. (original) The method of claim 22 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

24. (original) The method of claim 21 wherein the double stranded DNA dye is a primer-based double stranded DNA dye.

25. (original) The method of claims 24 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

26. (original) The method of claim 21 wherein the MT below the first T_m and/or the second T_m are 0.25 ^{O}C below, 0.5 ^{O}C below, 1.0 ^{O}C below, 1.5 ^{O}C below, or 2.0 ^{O}C below.

27. (original) The method of claim 21 wherein the MT above the first T_m and/or the second T_m are 0.25 ^{O}C above, 0.5 ^{O}C above, 1.0 ^{O}C above, 1.5 ^{O}C above, or 2.0 ^{O}C above.

28. (original) The method of claim 21 wherein the emission amount of the amplicons is obtained through a computer program performing the calculation of subtracting the pre-T_m emission from the post-T_m emission or subtracting the post-T_m emission from the pre-T_m emission.

29. (withdrawn) A method for real-time detecting and quantifying a total of *n* nucleic acid templates in a PCR mixture comprising the steps of:

a) thermally cycling a PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, nucleic acid templates including *n* nucleic acid templates, primers for amplifying *n* amplicons, and a double stranded DNA dye;

b) obtaining cycle by cycle a MT_k emission at MT_k and $MT_{(k+1)}$, wherein $T_{m(k-1)} < MT_k < T_{mk} < MT_{(k+1)} < T_{m(k+1)}$, T_{mk} is the T_m of a *k*th amplicon, $T_{m(k-1)}$ is the T_m of a (*k*-1)th amplicon except that $T_{m(k-1)}$ is an annealing and/or an extension temperature when k=1, $T_{m(k+1)}$ is the T_m of a (*k*+1)th amplicon except that $T_{m(n+1)}$ is a total denaturing temperature when k=n, and *k* and *n* are positive integers, $1 \leq k \leq n$, and $n \geq 2$;

c) determining cycle by cycle an emission amount of the *k*th amplicon which is the difference between the MT_k emission and the $MT_{(k+1)}$ emission.

30. (withdrawn) The method of claim 29 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

31. (withdrawn) The method of claim 30 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

32. (withdrawn) The method of claim 29 wherein the double stranded DNA dye is a primer-based double stranded DNA dye that is covalently linked to the primers.

33. (withdrawn) The method of claims 32 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-Fl.

34. (withdrawn) The method of claim 29 wherein $T_{m(k-1)}$ +0.25^{O}C< MT_k < T_{mk}, $T_{m(k-1)}$ +0.5^{O}C< MT_k < T_{mk}, $T_{m(k-1)}$ +1.0^{O}C< MT_k < T_{mk}, $T_{m(k-1)}$ +1.5^{O}C< MT_k < T_{mk}, or $T_{m(k-1)}$ +2.0^{O}C< MT_k < T_{mk}.

35. (withdrawn) The method of claim 29 wherein T_{mk} +0.25^{O}C< $MT_{(k+1)}$ < $T_{m(k+1)}$, T_{mk} +0.5^{O}C< $MT_{(k+1)}$ < $T_{m(k+1)}$, T_{mk} +1.0^{O}C< $MT_{(k+1)}$ < $T_{m(k+1)}$, T_{mk} +1.5^{O}C< $MT_{(k+1)}$ < $T_{m(k+1)}$, T_{mk} +2.0^{O}C< $MT_{(k+1)}$ < $T_{m(k+1)}$.

36 (withdrawn) The method of claim 29 wherein $T_{m(k-1)}$< MT_k < T_{mk}-0.25^{O}C, $T_{m(k-1)}$ < MT_k < T_{mk}-0.5^{O}C, $T_{m(k-1)}$< MT_k < T_{mk}-1.0^{O}C, $T_{m(k-1)}$< MT_k < T_{mk} –1.5^{O}C, or $T_{m(k-1)}$< MT_k < T_{mk}-2.0^{O}C.

37. (withdrawn) The method of claim 29 wherein T_{mk}< $MT_{(k+1)}$< $T_{m(k+1)}$-0.25^{O}C, T_{mk}< $MT_{(k+1)}$ < $T_{m(k+1)}$-0.5^{O}C, T_{mk}< $MT_{(k+1)}$ < $T_{m(k+1)}$-1.0^{O}C, T_{mk}< $MT_{(k+1)}$ < $T_{m(k+1)}$ - 1.5^{O}C, T_{mk}< $MT_{(k+1)}$ < $T_{m(k+1)}$-2.0^{O}C.

38. (withdrawn) The method of claim 29 wherein $T_{m(k-1)}$ + 0.25^{O}C< MT_k < T_{mk}-0.25^{O}C, $T_{m(k-1)}$ + 0.5^{O}C< MT_k < T_{mk} - 0.5^{O}C, $T_{m(k-1)}$ + 1.0^{O}C< MT_k < T_{mk} - 1.0^{O}C, $T_{m(k-1)}$ + 1.5^{O}C < MT_k < T_{mk} - 1.5^{O}C or $T_{m(k-1)}$ +2.0^{O}C< MT_k < T_{mk} - 2.0^{O}C.

39. (withdrawn) The method of claim 29 wherein $T_{mk} + 0.25^{O}C < MT_{(k+1)} < T_{m(k+1)} - 0.25^{O}C$, $T_{mk} + 0.5^{O}C < MT_{(k+1)} < T_{m(k+1)} - 0.5^{O}C$, $T_{mk} + 1.0^{O}C < MT_{(k+1)} < T_{m(k+1)} - 1.0^{O}C$, $T_{mk} + 1.5^{O}C < MT_{(k+1)} < T_{m(k+1)} - 1.5^{O}C$, or $T_{mk} + 2.0^{O}C < MT_{(k+1)} < T_{m(k+1)} - 2.0^{O}C$.

40. (withdrawn) The method of claim 29 wherein $2 \leq n \leq 35$, $2 \leq n \leq 18$, $2 \leq n \leq 10$, $2 \leq n \leq 7$, or $2 \leq n \leq 5$.

41. (withdrawn) The method of claim 40 wherein n = 2, 3, 4, or 5.

42 (withdrawn) The method of claim 29 wherein the PCR mixture further comprises a FRET based probe.

43. (withdrawn) The method of claim 42 wherein the FRET based probe is selected from the group consisting of a Taqman probe, a double-dye oligonucleotide probe, an Eclipse probe, a Molecular Beacon probe, a Scorpion probe, a Hybridization probe, a ResonSense probe, a Light-up probe, and a Hy-Beacon probe.

44. (withdrawn) The method of claim 29 wherein the PCR mixture further comprises a second primer-based double stranded DNA dye that emits differently from the double stranded DNA dye.

45. (withdrawn) The method of claim 29 wherein the emission amount of the *k*th amplicon is obtained through a computer program performing the subtraction of MT_k emission from $MT_{(k+1)}$ emission or the subtraction of the $MT_{(k+1)}$ emission from MT_k emission.

46. (withdrawn) A method for detecting and quantifying a total of *n* nucleic acid templates in multiplex real-time PCR comprising the steps of:

a) thermally cycling a PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, nucleic acid templates including *n* nucleic acid templates, primers for amplifying *n* amplicons, and a double stranded DNA dye;

b) obtaining cycle by cycle a pre-T_{mk} emission of the *k*th amplicon at a MT between $T_{m(k-1)}$ and T_{mk} and a post-T_{mk} emission of the *k*th amplicon at a MT between T_{mk} and $T_{m(k+1)}$, wherein $T_{m(k-1)} < T_{mk} < T_{m(k+1)}$, T_{mk} is the T_m of a *k*th amplicon, $T_{m(k-1)}$ is the T_m of a (*k*-1)th amplicon except that $T_{m(k-1)}$ is an annealing and/or an extension temperature when *k*=1, $T_{m(k+1)}$ is the T_m of a (*k*+1)th amplicon except that $T_{m(n+1)}$ is a total denaturing temperature when *k*=n, and *k* and *n* are positive integers, $1 \leq k \leq n$, and $n \geq 2$;

c) determining cycle by cycle an emission amount of the *k*th amplicon which is the difference between the pre-T_{mk} emission and the post-T_{mk} emission.

47. (withdrawn) The method of claim 46 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

48. (withdrawn) The method of claim 47 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

49. (withdrawn) The method of claim 46 wherein the double stranded DNA dye is a primer-based double stranded DNA dye.

50. (withdrawn) The method of claims 49 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

51. (withdrawn) The method of claim 46 wherein the MT between $T_{m(k-1)}$ and T_{mk} is $T_{m(k-1)}$ +0.25^{O}C< the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk}, $T_{m(k-1)}$ +0.5^{O}C< the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk}, $T_{m(k-1)}$ +1.0^{O}C< the MT between $T_{m(k-1)}$ and T_{mk}< T_{mk}, $T_{m(k-1)}$ +1.5^{O}C< the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk}, or $T_{m(k-1)}$ +2.0^{O}C< MT_k < T_{mk}.

52. (withdrawn) The method of claim 46 wherein the MT between T_{mk} and $T_{m(k+1)}$ is T_{mk} +0.25^{O}C< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$, T_{mk} +0.5^{O}C< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$, T_{mk} +1.0^{O}C< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$, T_{mk} +1.5^{O}C< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$, T_{mk} +2.0^{O}C< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$.

53. (withdrawn) The method of claim 46 wherein the MT between $T_{m(k-1)}$ and T_{mk} is $T_{m(k-1)}$< the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk}-0.25^{O}C, $T_{m(k-1)}$ < the MT between $T_{m(k-1)}$ and T_{mk}< T_{mk}-0.5^{O}C, $T_{m(k-1)}$< the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk}-1.0^{O}C, $T_{m(k-1)}$< the MT between $T_{m(k-1)}$ and T_{mk}< T_{mk} –1.5^{O}C, or $T_{m(k-1)}$< the MT between $T_{m(k-1)}$ and T_{mk}< T_{mk}-2.0^{O}C.

54. (withdrawn) The method of claim 46 wherein the MT between T_{mk} and $T_{m(k+1)}$ is T_{mk}< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$-0.25^{O}C, T_{mk}<the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$-0.5^{O}C, T_{mk}< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$-1.0^{O}C, T_{mk}< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$ -1.5^{O}C, T_{mk}<the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$-2.0^{O}C.

55.. (withdrawn) The method of claim 46 wherein the MT between $T_{m(k-1)}$ and T_{mk} is $T_{m(k-1)}$ + 0.25^{O}C<the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk}-0.25^{O}C, $T_{m(k-1)}$ + 0.5^{O}C<the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk} - 0.5^{O}C, $T_{m(k-1)}$ + 1.0^{O}C<the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk} - 1.0^{O}C, $T_{m(k-1)}$ + 1.5^{O}C <the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk} - 1.5^{O}C or $T_{m(k-1)}$ +2.0^{O}C< the MT between $T_{m(k-1)}$ and T_{mk} < T_{mk} - 2.0^{O}C.

56. (withdrawn) The method of claim 46 wherein the MT between T_{mk} and $T_{m(k+1)}$ is T_{mk} + 0.25°C< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$-0.25°C, T_{mk} + 0.5°C< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$- 0.5°C, T_{mk} + 1.0°C<the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$- 1.0°C, T_{mk} + 1.5°C < the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$- 1.5°C, or T_{mk} +2.0°C< the MT between T_{mk} and $T_{m(k+1)}$ < $T_{m(k+1)}$- 2.0°C.

57. (withdrawn) The method of claim 46 wherein $2 \leq n \leq 35$, $2 \leq n \leq 18$, $2 \leq n \leq 10$, $2 \leq n \leq 7$, or $2 \leq n \leq 5$.

58 (withdrawn) The method of claim 46 wherein the PCR mixture further comprises a FRET based probe.

59. (withdrawn) The method of claim 46 wherein the FRET based probe is selected from the group consisting of a Taqman probe, a double-dye oligonucleotide probe, an Eclipse probe, a Molecular Beacon probe, a Scorpion probe, a Hybridization probe, a ResonSense probe, a Light-up probe, and a Hy-Beacon probe.

60. (withdrawn) The method of claim 46 wherein the PCR mixture further comprises a second primer-based double stranded DNA dye that emits differently from the double stranded DNA dye.

61. (withdrawn) The method of claim 46 wherein the emission amount of the *k*th amplicon is obtained through a computer program performing the subtraction of the pre-T_{mk} emission from the post-T_{mk} emission or the subtraction of the post-T_{mk} emission from the pre-T_{mk} emission

62. (withdrawn) A computer software program for quantifying a real-time PCR amplicon which, when executed by a computer processor, performs the subtraction

of a pre-T_m emission from a post-T_m emission or the subtraction of the post-T_m emission from the pre-T_m emission.

63. (withdrawn) The computer software program of claim 62 wherein the emission was obtained from a double stranded DNA dye.

64. (withdrawn) The computer software program of claim 62 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

65. (withdrawn) The computer software program of claim 64 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

66. (withdrawn) The computer software program of claim 62 wherein the double stranded DNA dye is a primer-based double stranded DNA dye that is covalently linked to the primers.

67. (withdrawn) The computer software program of claim 66 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

68. (withdrawn) The computer software program of claim 62 wherein a pre-T_m emission is obtained at a MT below the T_m of the amplicon and a post-T_m emission is obtained at a MT above the T_m.

69. (withdrawn) The computer software program of claim 68 wherein the MT below the T_m is 0.25 ^{O}C below, 0.5 ^{O}C below, 1.0 ^{O}C below, 1.5 ^{O}C below, or 2.0 ^{O}C below the T_m.

70. (withdrawn) The computer software program of claim 68 wherein the MT above the T_m is 0.25 ^{O}C above, 0.5 ^{O}C above, 1.0 ^{O}C above, 1.5 ^{O}C above, or 2.0 ^{O}C above the T_m.

71. (withdrawn) The computer software program of claim 62 which is stored and/or executed in a PCR instrument.

72. (withdrawn) The computer software program of claim 62 which is stored and/or executed in a computer connected to a PCR instrument.

73. (withdrawn) A computer program product comprising a computer memory having a computer software program, wherein the computer software program, when executed by a computer processor, performs the subtraction of a pre-T_m emission from a post-T_m emission or the subtraction of the post-T_m emission from the pre-T_m emission.

74. (withdrawn) The computer program product of claim 73 wherein the emission was obtained from a double stranded DNA dye.

75. (withdrawn) The computer program product of claim 73 wherein the double stranded DNA dye is a double stranded DNA intercalating dye.

76. (withdrawn) The computer program product of claim 75 wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

77. (withdrawn) The computer program product of claim 73 wherein the double stranded DNA dye is a primer-based double stranded DNA dye that is covalently linked to the primers.

78. (withdrawn) The computer program product of claim 77 wherein the primer-based double stranded DNA dye is selected from the group consisting of fluorescein, FAM, JOE, HEX, TET, Alexa Fluor 594, ROX, and TAMRA, rhodamine, BODIPY-FI.

79. (withdrawn) The computer program product of claim 73 wherein a pre-T_m emission is obtained at a MT below the T_m of the amplicon and a post-T_m emission is obtained at a MT above the T_m.

80. (withdrawn) The computer program product of claim 79 wherein the MT below the T_m is 0.25 ^{O}C below, 0.5 ^{O}C below, 1.0 ^{O}C below, 1.5 ^{O}C below, or 2.0 ^{O}C below the T_m.

81. (withdrawn) The computer program product of claim 79 wherein the MT above the T_m is 0.25 ^{O}C above, 0.5 ^{O}C above, 1.0 ^{O}C above, 1.5 ^{O}C above, or 2.0 ^{O}C above the T_m.

82. (withdrawn) The computer program product of claim 73 which is stored and/or executed in a PCR instrument.

83. (withdrawn) The computer program product of claim 73 which is stored and/or executed in a computer connected to a PCR instrument.

84. (withdrawn) A PCR instrument comprising the computer program product of claim 73.